

WHAT IS CLAIMED IS:

1. An apparatus for separating zones of a density gradient, comprising:
a float having a concave upper surface, said float being adapted for insertion into
a vessel with a density gradient therein, said concave upper surface forming a well for
5 capturing a zone of the density gradient.

2. An apparatus as set forth in claim 1 further comprising a positioning
means for positioning the float within the density gradient for the removal of at least one
zone of the gradient.

3. An apparatus as set forth in claim 1 wherein said float is formed with a
10 convex lower surface.

4. An apparatus as set forth in claim 3, wherein said convex lower surface
has a conical shape.

5. An apparatus as set forth in claim 3, wherein said convex lower surface
has a spherical shape.

15 6. An apparatus as set forth in claim 1, wherein said concave upper surface
has a conical shape.

7. An apparatus as set forth in claim 6, wherein a central portion of said
concave upper surface has a spherical shape.

8. An apparatus as set forth in claim 1, wherein in response to downward
20 movement of said float into the density gradient, at least one zone is capturable within
said concave upper surface.

9. An apparatus as set forth in claim 1, wherein said float is made of a
material having a density lower than the density than any of the zones of the density
gradient such that said float is buoyant on an upper surface of the density gradient.

10. An apparatus as set forth in claim 9, wherein said float is made of a plastic material.

11. An apparatus as set forth in claim 2, wherein said positioning means includes a pipette contactable with a central portion of said concave upper surface.

5 12. An apparatus as set forth in claim 11, wherein movement of said pipette is controlled by a manually controlled mechanical positioning device.

13. An apparatus as set forth in claim 11 wherein movement of said pipette is controlled by a computer.

10 14. An apparatus as set forth in claim 11, wherein said pipette is connected to a means for selectively applying suction for removal of a captured zone of the density gradient.

15. An apparatus as set forth in claim 14, wherein said means for selectively applying suction is manually controlled by a microprocessor.

15 16. An apparatus as set forth in claim 1, wherein said concave upper surface is further formed with an annular groove for receiving a gripper.

17. A method for removing zones from a density gradient having a plurality of zones therein, the method comprising the steps of:

forming a density gradient in a vessel;

applying sample particles to the gradient;

20 centrifuging to separate the particles into one or more zones in the gradient;

inserting a float into the vessel, the float having a concave upper surface, the concave upper surface defining a well for capturing a zone of the density gradient;

pushing the float downward into the vessel such that at least a portion of one zone of the density gradient spills over an upper circumferential edge of the float into the well;

25 and

removing the captured zone from the well.

18. A method as set forth in claim 17, wherein:

said pushing step includes using a pipette to contact and push the float downward; and

5 said removing step includes applying suction to the pipette to remove the captured zone from the well.

19. A method as set forth in claim 17, further comprising the steps of:

pushing the float downward into the vessel such that at least a portion of a second zone of the density gradient spills over an upper circumferential edge of the float into the
10 well; and

removing the captured second zone from the well.

20. A method as set forth in claim 19, wherein:

said pushing step in claim 19 includes using a pipette to contact and push the float downward; and

15 said removing step in claim 19 includes applying suction to the pipette to remove the second captured zone from the well.

21. An apparatus for automatically separating and removing zones of a density gradient, comprising:

an X-Y manipulator device having a gripper;

20 a computer connected to said X-Y manipulator device for controlling position of said gripper;

a pipette positionable on said gripper;

a float having a concave upper surface, said float being adapted for insertion into a vessel with a density gradient therein, said concave upper surface forming a well for
25 capturing a zone of the density gradient.

22. An apparatus as set forth in claim 21 wherein said computer controls movement of said X-Y manipulator device and said gripper for positioning said float within the density gradient for the removal of at least one zone of the gradient.

23. An apparatus as set forth in claim 21 wherein said float is formed with a convex lower surface.

24. An apparatus as set forth in claim 23, wherein said convex lower surface has a conical shape.

25. An apparatus as set forth in claim 23, wherein said convex lower surface has a spherical shape.

26. An apparatus as set forth in claim 21, wherein said concave upper surface has a conical shape.

27. An apparatus as set forth in claim 26, wherein a central portion of said concave upper surface has a spherical shape.

28. An apparatus as set forth in claim 21, wherein in response to downward movement of said float into the density gradient, at least one zone is capturable within said concave upper surface.

29. An apparatus as set forth in claim 21, wherein said float is made of a material having a density lower than the density than any of the zones of the density gradient such that said float is buoyant on an upper surface of the density gradient.

30. An apparatus as set forth in claim 29, wherein said float is made of a plastic material.

31. An apparatus as set forth in claim 22, wherein said pipette is contactable with a central portion of said concave upper surface.

32. An apparatus as set forth in claim 31, wherein said pipette includes a means for selectively applying suction for removal of a captured zone of the density

gradient.

33. An apparatus as set forth in claim 32, wherein said means for selectively applying suction is controlled by said computer.

34. An apparatus for automatically separating and removing zones of a

density gradient, comprising:

a stationary pipetter;

a support table, said table being selectively moveable in vertical directions;

a vessel supported on said table;

a float having a concave upper surface, said float being inserted into said vessel

with a density gradient therein, said concave upper surface forming a well for capturing a zone of the density gradient; and

at least one separate vessel for receiving removed portions of the density gradient.

35. An apparatus as set forth in claim 34 wherein said float is formed with a convex lower surface.

36. An apparatus as set forth in claim 35, wherein said convex lower surface has a conical shape.

37. An apparatus as set forth in claim 35, wherein said convex lower surface has a spherical shape.

38. An apparatus as set forth in claim 34, wherein said concave upper surface has a conical shape.

39. An apparatus as set forth in claim 34, wherein in response to upward movement of said table, said float is partially submerged such that at least one zone is capturable within said concave upper surface.

40. An apparatus as set forth in claim 34, wherein said float is made of a

material having a density lower than the density than any of the zones of the density gradient such that said float is buoyant on an upper surface of the density gradient.

41. An apparatus as set forth in claim 34, wherein said float is made of a plastic material.

42. An apparatus as set forth in claim 34, wherein a tip portion of said pipette is contactable with a central portion of said concave upper surface.

43. An apparatus as set forth in claim 34, further comprising:

a concentric tube surrounding a portion of said pipette;

a pressure supplying means connected to said concentric tube;

a cap having an aperture for receiving said concentric tube and said pipette, said cap being fitted on an upper portion of said vessel thereby sealing said vessel with said pipette and said concentric tube extending into said vessel; and

wherein said pressure supplying means selectively supplies air pressure to said concentric tube thereby increasing air pressure within said vessel forcing any liquid in said concave upper surface of said float into said pipette for delivery to said separate vessel.

44. An apparatus as set forth in claim 43, further comprising:

a table supporting said separate vessel and a plurality of other separate vessels, said table being moveable to position one of said separate vessels under an outlet connected to said pipette for receiving liquid from said pipette.

45. An apparatus as set forth in claim 43, further comprising an o-ring in said cap providing a seal between said concentric tube and said cap.

46. An apparatus as set forth in claim 43, further comprising an o-ring on said cap providing a seal between said vessel and said cap.

47. An apparatus as set forth in claim 43, further comprising a clamp for

fixing said cap to said vessel.

48. An apparatus as set forth in claim 43, wherein movement of said table is controlled by a computer.

49. An apparatus for manipulating floats, comprising:

5 a pair of gripper jaws configured for insertion into a depression in a float, said pair of jaws being extendable way from one another for contact with inner surfaces of the float.

50. An apparatus for manipulating floats, as set forth in claim 49, wherein said gripper jaws are spring biased away from one another.

10 51. An apparatus for manipulating floats, as set forth in claim 50, wherein said gripper jaws are urged toward each other by a pair of rollers that contact outer surfaces of a portion of said gripper jaws.

52. An apparatus for manipulating floats, as set forth in claim 51, wherein said pair of rollers are moved up and down along said portion of said gripper jaws by a
15 positioning mechanism.

REFERENCES

Anderson, N.G., ed. The Development of Zonal Centrifuges. National Cancer
Institute Monograph 21, 1966

5

Price, C. A. Centrifugation in Density Gradients. Academic Press, N.Y. 1982

Scheeler, P. Centrifugation in Biology and Medical Science. John Wiley & Sons
N.Y. 1981

10

Anderson, N.G. A simple method for observing refractive index gradients in
liquids. Biochim Biophys Acta 25: 418, 1957

15

Anderson, N.G., Bond, H.E., and Canning, R.E. Analytical techniques for cell
fractions. I. Simplified gradient elution programming. Analyt Biochem 3: 472-478,
1962.

20

Anderson, N.G., and Rutenberg, E. Analytical techniques for cell fractions. A
simple gradient-forming apparatus. Anal Biochem 21: 259-265, 1967.

Candler, E.L., Nunley, C.E., and Anderson, N.G. Analytical techniques for cell
fractions. VI. Multiple gradient-distributing rotor (B-XXI). Anal Biochem 21: 253-258,
1967

25

Albright, J.F., and Anderson, N.G. A method for the rapid fractionation of

particulate systems by gradient differential centrifugation. Exptl Cell Research 15:
271-281, 1958

Anderson, N.G., Bond, H.E., and Canning, R.E. Analytical techniques for cell
5 fractions. I. Simplified gradient elution programming. Analyt Biochem 3: 472-478,
1962.

Fisher, W.D., G.B. Cline, and Anderson, N.G. Density gradient centrifugation in
angle-head rotors. The Physiologist 6: 179, 1963.

Anderson, N.G., and Rutenberg, E. Analytical techniques for cell fractions. A
10 simple gradient-forming apparatus. Anal Biochem 21: 259-265, 1967.

Candler, E.L., Nunley, C.E., and Anderson, N.G. Analytical techniques for cell
15 fractions. VI. Multiple gradient-distributing rotor (B-XXI). Anal Biochem 21: 253-258,
1967

Luthe DS A simple technique for the preparation and storage of sucrose
gradients. Anal Biochem 135:230-2, 1983

Hirst W, Cox RA A method for predicting the location of particles sedimenting
20 in sucrose gradients. Anal Biochem 131:51-68, 1983

Clark AG, Gellen JW Hydrostatically balanced gradient-formers: programming
25 of gradients. Anal Biochem 103:94-100, 1980

Olenick JG, Lorenz PE A floating device to permit fractionation of density
gradients from the top. Anal Biochem 97:72-76, 1979

5 Sartory WK, Halsall HB Design of a generalized n-solute mixing-chamber
gradient generator. Anal Biochem 88:539-551, 1978

McRee D Inexpensive apparatus for preparation of multiple discontinuous
gradient. Anal Biochem 87:638-652, 1978

10 Sheeler P, Doolittle MH, White HR Method and apparatus for producing and
collecting a multiplicity of density gradients. Anal Biochem 87:612-621, 1978

Michov BM A concentration gradient system. Anal Biochem 86:432-442, 1978

15 Corless JM Simple and inexpensive fabrication of small-volume density
gradients. Anal Biochem 84:251-255, 1978

Gordon J, Ramjouw HP A simple design of an apparatus for the generation of
20 sucrose gradients for large-scale zonal separation of ribosomal subunits. Anal Biochem
83:763-766, 1977

Gregor HD A new method for the rapid separation of cell organelles. Anal
Biochem 82:255-257, 1977

25

Khandjian EW In situ studies of subcellular particles immobilized in sucrose-acrylamide density gradients. *Anal Biochem* 77:387-396 1977

Allington RW, Brakke MK, Nelson JW, Aron CG, Larkins BA Optimum
5 conditions for high-resolution gradient analysis. *Anal Biochem* 73:78-92, 1976

Gasser KW, DiDomenico J, Hopfer U Separation of cell organelles in density
gradients based on their permeability characteristics. *Anal Biochem* 171:41-46, 1988

10 Shearer G Jr A syringe-based gradient former for linear and exponential
gradients. *Anal Biochem* 221:397-400, 1994

Graham J, Ford T, Rickwood D The preparation of subcellular organelles from
mouse liver in self-generated gradients of iodixanol. *Anal Biochem* 220:367-373, 1994

15 Ford T, Graham J, Rickwood D Iodixanol: a nonionic iso-osmotic
centrifugation medium for the formation of self-generated gradients. *Anal Biochem*
220:360-366, 1994

20 Davis PB, Pearson CK Characterization of density gradients prepared by
freezing and thawing a sucrose solution. *Anal Biochem* 91:343-349, 1978

Liedtke R, Mosebach KO An apparatus for density gradient forming and
nonpuncturing fractionation. *Anal Biochem* 62:377-385, 1974

25

McCarty KS Jr, Vollmer RT, McCarty KS Improved computer program data for the resolution and fractionation of macromolecules by isokinetic sucrose density gradient sedimentation. Anal Biochem 61:165-183, 1974

5 Lange CS, Liberman DF A semiautomated system for the production and analysis of sucrose density gradients. Anal Biochem 59:129-145, 1974

Bylund DB, Bruening G Prediction of centrifugation times for equilibrium and velocity sedimentation on various gradients. Anal Biochem 58:47-56, 1974

10

Sinclair JH Churchill L, Banker G, Cotman CW Gradient design to optimize rate zonal separations. Anal Biochem 56:370-382, 1973.

15

Hopkins TR Another density gradient fractionator. Anal Biochem 53:339-341,

1973

Atherton RS, Hawtin P, Hutchinson P Chromatography and zonal centrifugation. Prediction of the optimum initial chamber compositions of a multichambered concentration and density gradient device. Anal Biochem 49:326-335,

20

1972

Dingman CW A convenient program for the rapid calculation of sedimentation coefficients in linear salt or sucrose gradients. Anal Biochem 49:124-133, 1972

25

Leifer W, Kreuzer T Experiments and theoretical calculations for forming

gradients for zonal rotor centrifugation. Anal Biochem 44:89-96, 1971

Siakotos AN, Pennington K, McInnes A New loading system for preparing
density gradients for swinging-bucket rotors using programmed gradient pumps. Anal

5 Biochem 43:32-41, 1971

Neff SH, Meeker GL A modified fixed-volume mixer for extended sucrose
density gradients. Anal Biochem 41:365-371, 1971

10 Pretlow TG Estimation of experimental conditions that permit cell separations by
velocity sedimentation on isokinetic gradients of Ficoll in tissue culture medium. Anal
Biochem 41:248-255, 1971

15 Wallach DF A simple system for rapid generation of duplicate density gradients.
Anal Biochem 37:138-141, 1970

Shore SL, Phillips DJ, Reimer CB Preformed frozen sucrose gradients--a new
laboratory aid. Anal Biochem 31:114-117, 1969

20 Margolis J A versatile gradient-generating device. Anal Biochem 27:319-322,
1969

Henderson AR A constant-volume device for preparing isokinetic sucrose
density gradients. Anal Biochem 27:315-318, 1969.

25

Leif RC Density gradient system. II. A 50 channel programmable undulating diaphragm peristaltic pump. Anal Biochem 25:283-296, 1968

Leif RC Density gradient system. I. Formation and fractionation of density
5 gradients. Anal Biochem 25:271-282, 1968

Ayad SR, Bonsall RW, Hunt S A simple method for the production of accurate linear gradients using a constant-speed peristaltic pump. Anal Biochem 22:533-535, 1968.

10

Mach O, Lacko L Density gradient in a dextran medium. Anal Biochem 22:393-397, 1968

15

McCarty KS, Stafford D, Brown O Resolution and fractionation of macromolecules by isokinetic sucrose density gradient sedimentation. Anal Biochem 24:314-329, 1968

20

Birnie GD, Harvey DR A simple density-gradient engine for loading large-capacity zonal ultracentrifuge rotors. Anal Biochem 22:171-174, 1968

Anderson NG, Rutenberg E Analytical techniques for cell fractions. VII. A simple gradient-forming apparatus. Anal Biochem 21:259-265, 1967

25

Candler EL, Nunley CE, Anderson NG Analytical techniques for cell fractions. VI. Multiple gradient-distributing rotor (B-XXI). Anal Biochem 21:253-258, 1967

McEwen CR Tables for estimating sedimentation through linear concentration
gradients of sucrose solution. Anal Biochem 20:114-149, 1967

5 Gropper L, Griffith O Band-forming caps for the layering of sample in
swinging-bucket rotors. Anal Biochem 16:171-176, 1966

Samis HV Jr A simple density gradient generator. Anal Biochem 15:355-357,
1966

10

Camacho-Vanegas O, Loreni F, Amaldi F. Flat absorbance background for
sucrose gradients. Anal Biochem 228:172-173, 1995.

15

Smith GD, Osterloh KR, Peters TJ Computational analysis of density gradient
distribution profiles. Anal Biochem 160:17-23, 1987

Morand JN, Kent C A one-step technique for the subcellular fractionation of
total cell homogenates. Anal Biochem 159:157-162, 1986

20

Coombs DH, Watts NR Generating sucrose gradients in three minutes by tilted
tube rotation. Anal Biochem 148:254-259, 1985.

Samuels S., A continuous density gradient apparatus for use in zonal
ultracentrifuges. Anal Biochem 41:164-167, 1964.

25